

TESTING THE EVOLUTION OF INCREASED COMPETITIVE ABILITY (EICA) HYPOTHESIS IN A NOVEL FRAMEWORK

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Abstract. The “evolution of increased competitive ability” (EICA) hypothesis proposes that escape from natural enemies, e.g., after transcontinental introductions, alters the selection regime because costly defenses no longer enhance fitness. Such an evolutionary loss of defenses enables resources to be directed toward growth or other traits improving performance. We tested the EICA hypothesis in a novel framework in which the natural enemy is the traveler that follows its widespread host by accidental or deliberate (biocontrol) introductions. In a greenhouse experiment we used populations of *Senecio vulgaris* from North America, Europe, and Australia that differ in the history of exposure to the rust fungus *Puccinia lagenophorae*. Contrary to what is predicted by EICA, we found no evidence for increased levels of resistance to the rust fungus in plant populations with a longer history of rust exposure (Australia). Similarly, there was no evidence for reduced fecundity in these populations, although vegetative vigor, measured as secondary branching and growth rate, was lower. The maintenance of high rust resistance in populations with no (North America) or only a short history (Europe) of rust exposure is surprising given that resistance seems to incur considerable fitness costs, as indicated by the negative association between family mean resistance and fitness in the absence of disease observed for all three continents. The comparison of population differentiation in quantitative traits with estimates of differentiation in amplified fragment length polymorphic (AFLP) markers suggests that a number of fitness-related traits are under divergent selection among the studied populations. The proposed framework to test changes in the evolutionary trajectory underlying EICA can be employed in an expanded range of systems. These may include investigations on a cosmopolitan weed or crop when an antagonist is expanding its geographic range (such as our study), studies along a chronosequence of introduction time with expected increasing accumulation of natural enemies over time, or comparisons between introduced plant populations that differ in exposure time to biocontrol organisms.

Key words: common groundsel; differences in exposure time to a plant pathogen; evolution of increased competitive ability; EICA; fitness costs; molecular markers; *Puccinia lagenophorae*; resistance; rust fungus; selection differentials; *Senecio vulgaris*.

INTRODUCTION

Exposure to disease and herbivory over an extended period is expected to favor plant phenotypes that reduce either the amount of damage (resistance) or the negative effects of damage on plant fitness (tolerance) (Fineblum and Rausher 1995, Roy and Kirchner 2000, Stowe et al. 2000). Escape from natural enemies can occur after introductions to new areas, and resources normally lost to enemies or the production of defenses may be allocated to growth and/or reproduction by a plastic phenotypic response (“enemy release hypothesis”; Bazzaz et al. 1987, Thébaud and Simberloff 2001).

Alternatively, plants introduced into new areas may evolve reduced allocation to defense and increased allocation to growth and/or reproduction in the absence of enemies. Such evolutionary changes are only expected to occur if defense incurs a fitness cost, and plants that decrease investment in defense are at a selective advantage in the introduced range (“evolution of increased competitive ability” hypothesis or “EICA”; Blossey and Nötzold 1995). Some studies examining EICA have found a loss of defense in plants from introduced populations, but only a few have demonstrated altered resource allocation patterns that may favor growth and reproduction and ultimately facilitate demographic expansion of populations in the introduced range (cf. Bossdorf et al. 2005 and references therein). Most studies examining EICA have focused on the loss of defenses towards herbivory in introduced populations (Bossdorf et al. 2005). In this study we tested the EICA hypothesis from another viewpoint by comparing plant populations with a long history of enemy exposure with those in which the enemy has only been observed for a

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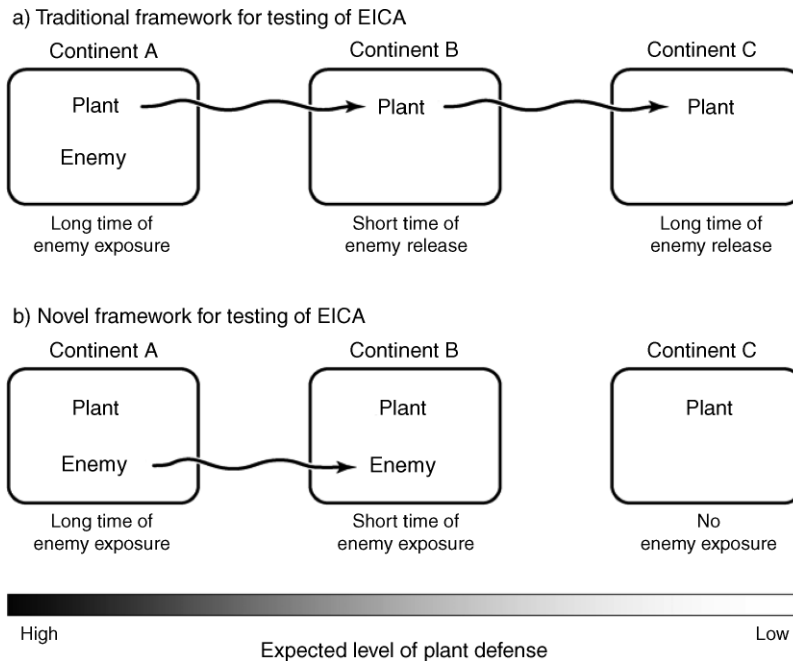


FIG. 1. (a) Traditional testing of the evolution of increased competitive ability (EICA) hypothesis and (b) the proposed novel framework offering an extended approach for testing this hypothesis. In (a), the plant is the traveler and escapes its natural enemies by invading new areas. In (b), the natural enemy is the traveler and follows its widespread host by accidental or deliberate (biocontrol) introductions.

limited time or is still absent (Fig. 1). This extended framework for testing changes in the evolutionary trajectory of fitness trade-offs with plant defense due to altered enemy exposure, i.e., the mechanism underlying EICA, is not limited to cross-continental comparisons in studies of biological invasion, in which plants have escaped their natural enemies, and thus opens up a range of testable systems.

Specifically we examined the role of selection history on defense in *Senecio vulgaris*, a cosmopolitan weed, against the host-specific rust fungus *Puccinia lagenophorae*, native to Australia and introduced to areas outside its native range. Thus, rather than focusing on loss of defense following enemy escape, we investigate the evolution of increased resistance and its consequences for growth and other performance traits due to an enemy expanding its geographical range.

One way to test the hypothesis of adaptive evolutionary change in response to selection by the pathogen is to compare offspring of plants from populations with different histories of infection in a common environment (Blossey and Nötzold 1995, Bossdorf et al. 2005). Genetic differentiation will be evident if, under these conditions, plant populations that differ in their time of exposure to rust infection diverge significantly in traits affecting defense and competitive ability. Some life-history and morphological variation may be due to one or a few loci (Gottlieb 1984), but most variation in complex traits is thought to be polygenic (Mitchell-Olds 1986) and requires a quantitative genetic approach in

order to separate genetic from environmental components of variation among populations. Quantitative genetic data can be combined with data from molecular markers, which, in the context of biological invasions, provide information about the origin and number of introduction and the amount of genetic variation introduced (cf. Bossdorf et al. [2005] for examples and further references). Here, we used neutral genetic markers to disentangle potential effects of divergent selection mediated by the rust fungus from other evolutionary mechanisms that could underlie patterns of population differentiation in quantitative traits that we observed in our common garden experiment (Merilä and Crnokrak 2001, Steinger et al. 2002). When selection is important and favors different phenotypes in populations of different regions, we would expect among-region variation in quantitative traits to exceed that of neutral molecular markers (Spitze 1993). Furthermore, we used genotypic selection analysis to examine potential divergent selection on phenotypic traits in rust-infected plants vs. noninfected plants. Such present-day patterns of selection were then compared with observed patterns of quantitative genetic differentiation among populations from three regions that differ in the time of occurrence of the rust fungus to infer the role of rust-mediated selection in evolutionary divergence.

Senecio vulgaris L. (Asteraceae), common groundsel, is a predominantly selfing annual plant that probably originated in southern Europe (Hull 1974, Kadereit

1984), but is now common worldwide, including North America and Australia, and is recognized as one of the world's most troublesome weeds (Holm et al. 1997). The autoecious rust fungus *Puccinia lagenophorae* (Uredinales, Basidiomycetes) is the most prevalent pathogen of *S. vulgaris* in Australia and Europe (Frantzen and Hatcher 1997, Wyss and Müller-Schärer 1999). *Puccinia lagenophorae* is understood to have first infected *S. vulgaris* in Australia and then spread to *S. vulgaris* in Europe in the early 1960s (Wyss and Müller-Schärer 1999 and references therein). Thus Australian populations of *S. vulgaris* have had the longest history of infection by the rust. European populations have a shorter association with the rust (~50 years), and at the time seeds were collected for this experiment *P. lagenophorae* was thought to be absent from North America. This system provides a framework in which to test EICA from another viewpoint. In this case Australian populations may be regarded as equivalent to the native range and predicted to have high levels of resistance. By analogy, North American plants would be considered introduced and invasive, having escaped infection, and are predicted to have low resistance but high vigor in the absence of selection by the pathogen. European populations have an intermediate exposure time to the pathogen and might be expected to have intermediate levels of resistance if selection history is important (Fig. 1).

Puccinia lagenophorae infection is expected to be an important selective agent due to its often high prevalence and strong impact on fitness caused by rapid and high levels of disease severity. It infects leaves, stems, and capitula of *S. vulgaris*, by way of typical orange-colored aeciospores, and can cause severe malformations (Wyss and Müller-Schärer 1999). Based on previous work we expect to find a strong differentiation among populations of different origin that may have been caused by selection due to differences in the length of exposure to the pathogen. Results from various studies on *S. vulgaris* and its antagonists indicate pronounced effects of disease and herbivory on plant growth and reproduction (e.g., Paul and Ayres 1987, 1990, Tinney et al. 1998), a suite of compensatory mechanisms (Paul and Ayres 1984, 1986, Obeso and Grubb 1994, Inglese and Paul 2006), genetic variation within and among populations (Müller-Schärer and Fischer 2001, Steinger et al. 2002), and genetic variation in resistance to pathogens, including *Erysiphe fischeri* and *P. lagenophorae* (Bevan et al. 1993a, b, c, Leiss and Müller-Schärer 2001). Resistance to *P. lagenophorae* in *S. vulgaris* has been found to show a continuous range of variation, and no gene-for-gene relationship between host resistance and pathogen virulence has been found. Therefore, resistance is believed to be nonspecific to race and quantitative in this system (Wyss and Müller-Schärer 1999).

Here we address the hypotheses that (1) plants of different origin and thus of different exposure time to

the rust pathogen differ in resistance and vigor according to the EICA hypothesis, i.e., plants of Australian origin have comparatively high resistance but low vigor; (2) there is an allocation cost of resistance that can explain low levels of resistance in the absence of rust infection; (3) different plant phenotypes are favored by selection in environments with different exposure time to rust infection, which can help explain the differences between origins; and (4) observed regional differences in quantitative traits can partially be explained by differential selection, rather than only due to founder effects and genetic drift.

METHODS

Study populations

The plants used in our study originated from seeds collected from populations of *Senecio vulgaris* subsp. *vulgaris* var. *vulgaris*, growing in apple orchards, thus minimizing differences in selection regime other than through infection by *Puccinia lagenophorae*. Seeds were sampled from three regions during 2002: Australia, with the longest history of infection; Europe (Switzerland), with an intermediate exposure time of ~50 years; and North America, where *P. lagenophorae* had not been reported at the time of seed collection (see Appendix A for a description of the study populations). In each region, seeds were collected from ~30 individual plants randomly selected from eight populations that were a minimum of 1 km apart. To minimize maternal effects, plants were grown for one generation in a heated glasshouse and selfed, and the resulting seeds, derived from a single seed per field-collected family, were used in this experiment.

In this study only the asexual reproductive cycle of *P. lagenophorae* was used, which produces the characteristic orange-colored aeciospores, typically between 10 and 14 days after inoculation with an aeciospore suspension. The systemic spread of infection is possible but dispersal of aeciospores from sporulating sori is the predominant mode of spread and disease build-up. The rust line used was collected from a *S. vulgaris* population in Switzerland and multiplied in the greenhouse on this Swiss *S. vulgaris* population. Ongoing molecular analyses (random amplified polymorphic DNA [RAPD], amplified fragment length polymorphism [AFLP]) of rust lines isolated from various European populations show a striking genetic uniformity both within and among populations (H. Müller-Schärer, *unpublished data*) suggesting absence or rare occurrence of sexual reproduction in Europe.

Experimental design

We randomly selected 10 seed families of *S. vulgaris* from each of eight populations from the three regions (240 plant families). Twenty seeds from each plant family were sown into 9 cm diameter pots filled with nutrient-amended peat (TKS 2, Floragard, Oldenburg, Germany). Seeds were moistened with a fine spray and

TABLE 1. Summary of hierarchical ANOVA (proc MIXED in SAS) in traits of *Senecio vulgaris* for resistance to rust (rust treatment only) and six other phenotypic traits (using no-infection treatment only), from three regions: North America, Europe (Switzerland), and Australia.

Trait	Region, F	Population, χ^2	Family, χ^2
No. capitula	2.67	15.61***	37.47***
Vegetative dry mass	0.04	6.56**	206.75***
Secondary branches	4.83*	14.40***	29.89***
Apical height	0.54	39.31***	59.53***
Reproductive allocation	2.85	19.83***	1.54
Growth rate	3.48*	34.61***	11.75***
Resistance	1.25	4.69*	16.86***

Note: Region was treated as a fixed effect (all $df = 2, 21$), whereas population (within region) and family (within population) were treated as random effects ($df = 1$; see *Methods*).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

kept on waterlogged capillary matting. When sufficient seedlings from all plant families were available, six of each were transplanted to individual 9 cm diameter pots filled with TKS 2. Three seedlings from each family were assigned to the infection treatment with *P. lagenophorae* and three to the control group (without infection). The experimental design can be summarized as 3 regions \times 8 populations \times 10 families \times 2 treatments \times 3 replicates. A rust-infected and control plant for each family were assigned to random positions on each of three glasshouse benches corresponding to the three replicates of each family \times treatment combination. Due to lack of space we used a fourth bench on which we placed an infected and noninfected pair of plants of randomly selected families. This resulted in a balanced incomplete block design. The inoculation of plants with the rust fungus was carried out when plants had between six and eight true leaves. Inoculations were made with an aqueous suspension of *P. lagenophorae* aeciospores (50 mg of rust spores in 100 mL of distilled water per 100 plants) applied with a de Vilbiss sprayer (de Vilbiss, Glendale Heights, Illinois, USA). After inoculation individual plants were covered in plastic bags to allow for a 12-h dew period. Plants in the control group were sprayed with distilled water only and covered with plastic bags for the same period. A second inoculation was carried out five days later.

Data collection

Three weeks after inoculation with rust spores the leaves of all plants were counted and a visual estimate of infection of all leaves of the inoculated plants was made using a 0–6 scale (0, no disease; 1, 0–5%; 2, 6–25%; 3, 26–75%; 4, 76–95%; 5, >95% of leaf surface with necrosis; 6, leaf dead). The total necrotic leaf area was calculated as a percentage of a whole plant using the formula $(2.5n_1 + 15n_2 + 50n_3 + 85n_4 + 95.5n_5 + 100n_6)/N$ where n_x is the number of leaves with rating x and N is the total number of leaves (Pfister and Défago 1998). No infection on control plants was observed at this time.

Plants were harvested when at least five flower heads, on each plant, had set seed. This was achieved by harvesting over 14 days and following the same sequence used as the blocks were started. At harvest we measured seven phenotypic traits (cf. Table 1) including the number of flowers and seed heads (number of capitula), dry mass of the vegetative (vegetative dry mass) and reproductive parts of the plant, the number of secondary branches, and the height of the plant from the soil surface to the apical meristem (hereafter, apical height). Growth rate was calculated as the change in the number of leaves between inoculation and disease assessment. Initial leaf number was used as a covariate in the analysis of growth rate. Reproductive allocation was calculated as the proportion of total shoot dry mass accounted for by the dry mass of reproductive parts. We followed the convention in the ecological literature (Stowe et al. 2000) and consider resistance to be made up of characters that reduce the amount of damage (Burdon 1987, Strauss and Agrawal 1999, Tiffin and Inouye 2000). We define damage as the proportion of leaf area infected by *P. lagenophorae* and resistance as 1 minus damage (Pilson 2000).

Data analysis, glasshouse experiment

We used hierarchical random effects ANOVA as implemented in the SAS software package (proc MIXED; SAS Institute 1989) to test for differences in trait means and to estimate components of variance in quantitative traits attributable to genetic and environmental sources. To determine the statistical significance of the random factors, population within region and family within population, in mixed model ANOVA, the -2 residual log-likelihood for the full model and a model that did not include the factor in question were compared. The difference in the residual log-likelihood was compared with a χ^2 distribution using a table value at twice the nominal significance level (e.g., by using $\alpha = 0.10$ critical values to conduct a 0.05-level test; Littell et al. 1996:44). The degree of population differentiation in phenotypic traits was represented by Q_{ST} , the proportion of total genetic variance that is among populations. For completely selfing species $Q_{ST} = \sigma_{pop}^2 / (\sigma_{pop}^2 + \sigma_{fam}^2)$, where σ_{pop}^2 is the among-population variance component and σ_{fam}^2 is the among-family (within-population) variance component (Bonnin et al. 1996).

To examine pathogen-mediated selection on plant phenotypic traits, we calculated genotypic selection differentials separately for each infection treatment (infected and control) based on family means of each trait. Linear selection differentials were calculated for each trait as the covariance between relative fitness (number of capitula divided by mean number of capitula within a given infection treatment) and trait values (family means) (Lande and Arnold 1983, Rausher 1992). Selection differentials were standardized by dividing the covariance by the standard deviation. Block effects were removed prior to analysis. Analysis of covariance was

used to test for differences in selection differentials between the rust infection and the no-infection treatments.

DNA extraction and AFLP analysis

All plant lines used in the glasshouse experiment were analyzed using the AFLP technique (Vos et al. 1995). DNA was extracted from fresh leaf tissue and AFLP analysis was carried out essentially as described by Haldimann et al. (2003). However, after preselective polymerase chain reaction (PCR) amplification, fragments were amplified in a second selective multiplex PCR amplification by combining the fluorescent-labeled 5-FAM (*EcoRI* + AGG) (Microsynth, Balgach, Switzerland), JOE (*EcoRI* + ACC) (Microsynth), and TAMRA (*EcoRI* + AGC) primers with the *MseI* + CAA primer in a first reaction and then with the *MseI* + CTT primer in a second reaction. The total reaction volume of 20 μL contained 0.05 $\mu\text{mol/L}$ of each of the three labeled *EcoRI* + ANN primer (a 1:1 mixture of labeled and unlabeled *EcoRI* + AGG primer was used to equalize fluorescence detection among dyes), 0.25 $\mu\text{mol/L}$ of *MseI* + CNN primer, 0.2 mmol/L of each dNTP (2'-desoxyribonukleosid-5'-triphosphat; Amersham Pharmacia Biotech Europe, Freiburg, Germany), 1 \times QIAGEN PCR buffer (QIAGEN, Valencia, California, USA), 2.25 mmol/L MgCl_2 , 0.5 units *Taq* DNA polymerase, and 3 μL of the diluted preselective products. Preliminary tests showed that the primers chosen produce highly reproducible AFLP patterns. Amplified fragments were separated by capillary electrophoresis with an ABI Prism 310 Genetic Analyzer using GeneScan-500 ROX as an internal size standard (Applied Biosystems, Foster City, California, USA). Electropherograms were scored for the presence (1) or absence (0) of fragments in the range 100–500 base pairs (bp) using GelComparII computer software (Applied Maths BVBA, Sint-Martens-Latem, Belgium).

Data analysis, AFLP markers

The 0/1 matrix obtained from scoring the AFLP markers was subject to analysis of molecular variance (AMOVA) using the software package ARLEQUIN (version 2.0; Schneider et al. 2000). AMOVA is based on pairwise squared Euclidean distances among AFLP phenotypes and allows the molecular variance to be partitioned among several hierarchical levels, analogous to conventional ANOVA (Excoffier et al. 1992). The significance of variance components at each hierarchical level is tested with a permutation procedure. From the variance components a Φ_{ST} statistic can be calculated, which is a good estimator of among-population genetic diversity, F_{ST} . Here, Φ_{ST} is defined as the proportion of total molecular variance that is due to differences among the units of a given level. We partitioned the total variance into components attributable to differences among regions (North America, Australia, and Europe),

among populations within regions, and among individuals within populations.

If AFLP markers can be considered effectively neutral, then the estimate of proportion of diversity among populations for the molecular data can be used as the null hypothesis for testing among-population variance for genetic traits. A Q_{ST} value significantly greater than the F_{ST} value for a type of character indicates diversifying selection, whereas a significantly smaller value will indicate that selection may be constraining among-population divergence caused by genetic drift (Spitze 1993).

RESULTS

Plant origin and genetic differentiation in performance and resistance

Experimental rust infection had a highly significant main effect, reducing growth and reproduction in all plants (Fig. 2, Appendix B). Plants from the three regions responded similarly to rust infection, as indicated by the nonsignificant treatment \times region interactions for most traits except reproductive allocation ($P < 0.05$; Appendix B).

In the no-infection treatment there was significant variation among plants from the three regions in growth rate and secondary branching (Table 1, Fig. 2). Plants from Australia produced fewer secondary branches than plants from Europe and North America (Tukey post hoc comparisons; $P < 0.05$) and they had a lower growth rate than plants from Europe ($P < 0.05$), but not North America ($P < 0.24$; Fig. 2).

Resistance did not vary among plants from different regions (Table 1). There was significant variation in resistance among populations and among families within populations (Table 1), which indicates the presence of genetic variation in resistance within and among populations.

Costs of resistance

To assess the costs of resistance we first determined that genetic variation for resistance existed among the plant families studied (see *Methods: Data analysis, glasshouse experiment*). We then tested for a significant genetic correlation between resistance (measured in the presence of the pathogen) and fitness in the absence of disease (Simms and Rausher 1987, Mauricio 1998, Rausher 2001). Compared to families that were susceptible to the rust, families with comparatively high resistance to rust infection produced fewer capitula in the no-infection treatment, indicating that resistance, or some trait with which it is correlated, is costly in the absence of disease. When populations from all regions are considered together there was a highly significant negative relationship between resistance and fitness (number of capitula) in the no-infection treatment (selection differential: -0.08 , $P < 0.001$); this was also significant when considered separately for plants from North America (-0.11 , $P < 0.001$) and Australia (-0.06 ,

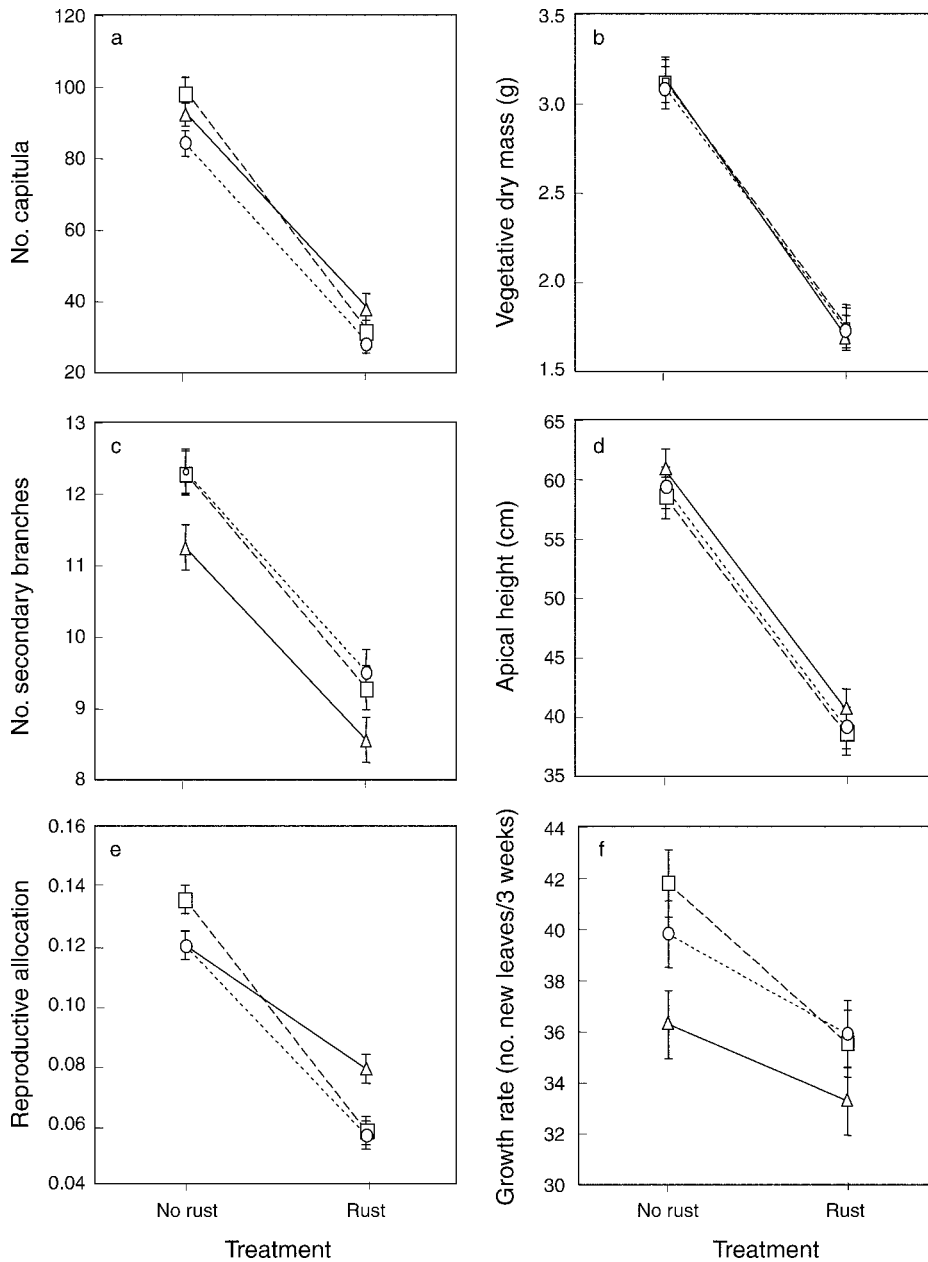


FIG. 2. Effect of rust (*Puccinia lagenophorae*) infection on traits of *Senecio vulgaris*: (a) number of capitula, (b) vegetative dry mass, (c) secondary branches, (d) apical height, (e) reproductive allocation (as a proportion), and (f) growth rate (mean \pm SE). Different symbols denote region least-square means in the rust infection and no-infection treatments (North America [circles], Europe [Switzerland; squares], Australia [triangles]).

$P = 0.04$) and but not significant in Europe (-0.05 , $P = 0.12$; Fig. 3). Analysis of covariance to test this relationship for differences between the regions was not significant ($F_{2,212} = 2.30$, $P = 0.103$). Plants with high growth rate after inoculation may receive low damage scores but have high fecundity. We thus used growth rate as a covariate in the analysis to correct for differences in growth rate but this had little influence on the relationship between resistance and fitness.

Among-population variation in AFLP markers and in quantitative traits

We scored a total of 178 AFLP markers of which 143 (80%) were polymorphic. Populations showed a moderate degree of spatial structure ($\text{Pop-}\Phi_{\text{ST}} = 0.24$) and the region of origin explained little of the total molecular variance ($\text{Reg-}\Phi_{\text{ST}} = 0.06$; estimated from AMOVA; Appendix C).

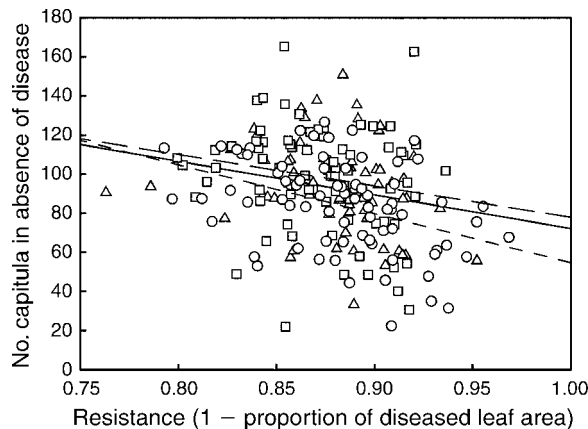


FIG. 3. Scatterplot of family mean number of capitula per plant in the absence of disease (no-infection treatment) vs. resistance in diseased plants (rust infection treatment). Corresponding standardized directional selection differentials (with significance) are: North America (circles, short-dashed line), -0.11 ($P = 0.0003$); Europe (Switzerland; squares, long-dashed line), -0.05 ($P = 0.12$); Australia (triangles, solid line), -0.06 ($P = 0.04$); overall, -0.08 ($P < 0.0001$).

We detected a significant among-region component of variation in two out of seven quantitative genetic traits, secondary branching and growth rate, with Q_{ST} for these traits exceeding the $Reg-\Phi_{ST}$ (Table 2). We also detected among-population variation in all measured quantitative traits although in some regions this variation was not significant (Table 2). Estimates of $Pop-Q_{ST}$ were relatively high, ranging from 0.05 for resistance in European plants to 0.70 for reproductive allocation in North American plants. Number of capitula, apical height, reproductive allocation, and growth rate exceeded $Pop-\Phi_{ST}$ estimates based on AFLP markers in all regions. Population differentiation in quantitative traits was generally larger in plants from

North America than in European plants, which was generally larger than in Australian plants.

Directional selection on plant traits

We calculated genotypic selection differentials for rust-infected and noninfected plants to examine whether rust infection alters patterns of selection on plant phenotypes. We report only linear selection differentials, as nonlinear (quadratic) differentials were in no case significant. Linear selection differentials were positive and significant for all traits in both the rust infection and the no-infection treatments (Table 3), indicating that selection favored families with higher values of the measured traits.

Directional selection on secondary branching and reproductive allocation differed in strength, but not direction, between plants grown in the rust infection and the no-infection treatments (Table 3).

DISCUSSION

Our study examined the predictions of the EICA hypothesis, using the novel framework provided by the plant–pathogen system of *S. vulgaris* and *P. lagenophorae*. We found significant genetic variation for resistance in *S. vulgaris* plants from three regions with different histories of infection by *P. lagenophorae* and costs of resistance in terms of reduced fecundity in the absence of disease. Costs of resistance were not always detected in previous studies (Bergelson and Purrington 1996, Strauss et al. 2002). Costs can be associated with qualitative resistance such as the presence of disease resistance (*R*) genes that conform to a gene-for-gene interaction (Korves and Bergelson 2004), but resistance in *S. vulgaris* is thought to be quantitative (Wyss and Müller-Schärer 1999), which is supported by our findings since all plant families became infected and variation in levels of resistance was continuous.

TABLE 2. Estimates of among-population variation in quantitative genetic traits (Q_{ST}) of *Senecio vulgaris*.

Trait	Region	Population		
		North America	Europe	Australia
No. capitula	0.09	0.33**	0.31**	0.22
Vegetative dry mass	0.00	0.14*	0.18*	0.07
Secondary branches	0.18*	0.47**	0.32**	0.11
Apical height	0.00	0.57***	0.25*	0.22*
Reproductive allocation	0.18	0.70***	0.57*	0.53*
Growth rate	0.18*	0.61***	0.46**	0.66***
Resistance	0.01	0.26*	0.05	0.19
Mean over traits	0.07	0.35	0.24	0.25
AFLP markers (Φ_{ST})	0.06***	0.24***	0.17***	0.15***

Notes: Q_{ST} values were calculated from variance components estimated with hierarchical ANOVA models (see *Methods*). Region refers to estimates of quantitative genetic differentiation at the level of regions, for all populations considered together. Other columns refer to estimates of quantitative genetic differentiation at the level of populations within the respective regions. Tests of significance were made using the difference in residual log-likelihood between models with and without population (see *Methods*). Corresponding estimates of population differentiation for amplified fragment length polymorphism (AFLP) markers are given.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3. Linear selection differentials based on family means, for vegetative traits of *Senecio vulgaris* in plants grown in rust infection and no-infection treatments (pooled over all regions).

Traits	Infected	Noninfected	Infected vs. noninfected	
			<i>F</i>	<i>P</i>
Vegetative dry mass	0.25***	0.18***	0.51	0.48
Secondary branches	0.21***	0.13***	12.42	<0.001
Apical height	0.16***	0.08***	1.06	0.31
Reproductive allocation	0.31***	0.06***	11.24	0.001
Growth rate	0.16***	0.10***	2.68	0.10

Notes: The “Infected vs. noninfected” columns indicate whether selection differentials differed significantly between the rust-infected and the noninfected plants by a 1-df contrast in ANCOVA (pooled over all regions, denominator df = 432). Boldface type indicates statistically significant differences ($P < 0.05$).

*** $P < 0.001$.

Since we found strong evidence that resistance to infection by *P. lagenophorae* is costly for *S. vulgaris*, we might therefore expect naïve plants with lower resistance to be especially common in North America. However, we found no significant difference in resistance between plants with different histories of rust infection. This is despite significant among-population and among-family variation in resistance, suggesting that there is genetic variation for resistance within the populations sampled. An explanation for this finding may be that resistance to *P. lagenophorae* is correlated with defense against other enemies (cf. Hallett et al. 1990, Hallett and Ayres 1992, Bevan et al. 1993b), which are present in the areas where *P. lagenophorae* was absent and selection for resistance against enemies in general has been maintained. The larger differentiation in resistance among North American populations compared to Europe and Australia could be explained by larger spatial variation in selection by native enemies or by the chronosequence of *S. vulgaris* introductions leading to variable loss of resistance (Siemann et al. 2006).

Improved competitive ability through a genetic shift in allocation from defense to growth as predicted by the EICA hypothesis has been tested in a number of previous studies, mainly using plant–herbivore systems (see Bossdorf et al. 2005 for a review). Improved performance has been observed in several cases (Blossey and Nötzold 1995, Siemann and Rogers 2001, 2003, Stastny et al. 2005), but not in others (Willis et al. 2000, Vilà et al. 2003), and in some cases invasive populations have even shown decreased competitive ability (Bossdorf et al. 2004). A number of studies have also shown loss of enemy resistance in introduced populations (e.g., Maron et al. 2004a, reviewed in Bossdorf et al. 2005). However, concurrent confirmation of both increased susceptibility to herbivory and higher competitive ability in the introduced range, the two conditions required to comply unequivocally to EICA, have so far only been found in four species (*Lythrum salicaria*, *Sapium sebiferum*, *Silene latifolia*, and *Solidago gigantea*; cited in Dietz and Edwards 2006).

In line with the EICA hypothesis, our analysis revealed decreased vigor of plants that have had a long

history of infection by *P. lagenophorae*. We found significant variation between regions in growth rate and secondary branching, and in both cases plants from Australia showed lower levels of vigor than plants from North America and Europe. Growth rate and secondary branching are likely to be important in determining the outcome of competition. Secondary branching in our study resulted in bushier plants with no loss in height. However, contrary to expectations of EICA, plants from North America and Europe did not differ in numbers of capitula from plants of Australian origin and thus did not show increased reproductive output.

Although there is evidence of considerable within-population genetic variation in fitness-related traits, this variation is not consistently related to the origin of the plants and therefore to the history of pathogen-induced selection. The high level of among-population variation we detect in our analyses suggests that there may be large differences between populations within origins in the direction or intensity of selection on all measured traits. If *P. lagenophorae* infection is an important selective force, it is possible that spatial and temporal heterogeneity of rust infection, in Australian and European populations, may maintain high genetic variation in defensive traits. The response to selection on traits in one environment may be impeded by gene flow from another environment in which selection differs or by alternating episodes of selection that might occur in different generations.

A number of previous studies of quantitative traits in native and introduced populations have demonstrated some sort of genetic divergence among plant populations (Bossdorf et al. 2005). Rapid evolutionary change therefore appears to be fairly common in plant invasions and introductions, and enemy release is expected to be one of the causal agents (Müller-Schärer et al. 2004). One of the objectives of this study was to explore the role of diversifying selection in population differentiation in quantitative traits between regions differing in exposure time to a natural enemy. The appropriate null hypothesis for such a test is not a complete absence of population subdivision, because random genetic drift and mutations are equally likely to cause populations to

diverge, as is selection. Although we collected our seed material from plants growing in apple orchards (with two exceptions; cf. Appendix A) to minimize differences in the selection regime other than through the rust fungus, we cannot rule out rapidly evolved divergent adaptations to broad-scale environmental conditions among the three regions (Maron et al. 2004b) that might explain our observed differences in branching pattern and growth rate. The higher Q_{ST} in these traits compared with Φ_{ST} suggests that selection at least partially contributed to the observed divergence among regions (Table 2).

Our study revealed moderate overall population differentiation ($\Phi_{ST} = 0.24$) in molecular markers (AFLP). Our estimate of the degree of population differentiation for Europe/Switzerland ($\Phi_{ST} = 0.17$; Table 2) is similar to the differentiation among populations collected in vineyards in Switzerland at a similar geographical scale ($\Phi_{ST} = 0.20$; Haldimann et al. 2003). It is lower than the mean G_{ST} value reported for widespread selfers ($G_{ST} = 0.46$; Hamrick and Godt 1996) and may indicate higher-than-expected gene flow among populations or considerable outcrossing in *S. vulgaris*. In the current study we did not control for the geographic scale but controlled for the habitat effect by including mostly populations from apple orchards. Indeed, the mean distance among populations differs among regions (Europe, 73 km; Australia, 246 km; and North America, 1983 km), and this may partly explain the higher Φ_{ST} (and Q_{ST}) for North America (Table 2). However, a correlation between pairwise genetic distances with geographic distances, separately for the three regions, revealed no indications for isolation by distance (Mantel test; Europe, $r = 0.03$, $P = 0.40$; Australia, $r = 0.25$, $P = 0.66$; North America, $r = 0.01$, $P = 0.44$). The observed differences in population differentiation among the regions, therefore, cannot be explained simply by their different sampling areas, as the larger among-population distances in North America apparently did not significantly constrain gene flow. Limited gene flow is expected to lead to rapid population differentiation, especially in selfing species with strongly fluctuating population sizes such as *S. vulgaris*.

Differentiation among populations in the two introduced ranges in North America and Australia may have been shaped further by their introduction history. However, the observed low differentiation among regions in AFLP markers together with the high molecular diversity in North America (not shown) suggests multiple introductions.

The evidence from our directional selection analysis suggests that in the presence of rust infection, selection is stronger for secondary branching and reproductive allocation compared with non-diseased plants (Table 3). These results are not in line with our observed differences among regions, where, e.g., secondary branching was found to be reduced in the plants from

Australia with an extended history of rust infection, as compared to plants from North American and European origin with no or short infection history. It therefore seems that selective agents other than rust infection may have contributed to genetic divergence in phenotypes.

In summary, we used a plant–pathogen system that has rarely been examined in the context of EICA and attempted to address many aspects of the hypothesis discussed and proposed in Bossdorf et al. (2005). Despite finding high levels of genetic variation for traits associated with growth and reproduction, and a high cost of resistance, we found no evidence of decreased fecundity or increased levels of resistance in populations that have had a long history of infection. In such populations, however, we found decreased vigor in terms of growth rate and secondary branching, which is in accordance with EICA predictions.

Our study has used an alternative framework to test changes in the evolutionary trajectory of fitness trade-offs with plant defense due to altered enemy exposure, i.e., the mechanism underlying the EICA hypothesis. Only a few studies so far have explored how differences in natural enemy loads among introduced populations may affect the potential for evolutionary change of selected plant traits after introduction. Using herbarium specimens of the invasive European weed *Pastinaca sativa* in North America through 152 years, Zangerl and Berenbaum (2005) found an increase in toxic furanocoumarins coincident in time with the accidental introduction of a major European herbivore in 1869. Interestingly, concentrations of furanocoumarins during earlier stages of establishment in North America (1850–1889) were even lower than in European specimens collected before 1889. Similarly, Siemann et al. (2006) studied herbivory in the Chinese tallow tree *Sapium sebiferum* along a chronosequence of introductions into North America and found that herbivory was highest and tree performance poorest where *S. sebiferum* has been introduced earlier, indicating that release from insect herbivores might have contributed to early invasion success, but accumulation of insect herbivores has apparently reduced this benefit over time.

Here, we put these studies in a novel conceptual framework and exemplify it with our study. This framework widens the range of systems useful to test EICA beyond examples from transcontinental comparisons in which plants have escaped their natural enemies, e.g., investigations on a cosmopolitan weed or crop, studies along a chronosequence of introduction time, or comparisons between introduced plant populations that differ in exposure time to biological control organisms. These systems could be used to test whether the presumed evolutionary shift toward reduced plant defense and increased vigor among plant invaders in the introduced range could be reversed by the geographic spread of a natural enemy (our study), the accumulation over time of natural enemies present in the introduced range (cf. Siemann et al. 2006), or when

biocontrol organisms are introduced, respectively (Müller-Schärer et al. 2004). Such cases have only rarely been considered when testing EICA (but see Vilà et al. 2003, Joshi and Vrieling 2005, Zangerl and Berenbaum 2005).

ACKNOWLEDGMENTS

We thank Nilgun Sailer for her extensive help during the course of these experiments, Nigel Paul for the many stimulating discussions, and the various seed collectors in North America and Australia. This study was financially supported by the Swiss National Science Foundation (grant number 3100-065356 to H. Müller-Schärer).

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APPENDIX A

A table showing the locations of study populations of *Senecio vulgaris* (*Ecological Archives* E089-022-A1).

APPENDIX B

A summary of hierarchical mixed-model ANOVA in growth and reproductive traits of *Senecio vulgaris* (*Ecological Archives* E089-022-A2).

APPENDIX C

A summary of hierarchical analysis of molecular variance (AMOVA) for 249 *Senecio vulgaris* individuals representing 24 populations from three regions: North America, Europe (Switzerland), and Australia (*Ecological Archives* E089-022-A3).

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Appendix A (Table A1). A table showing the location of study populations of *Senecio vulgaris*.

Population	Locality	Longitude (east)	Latitude (north)
North America			
CA1	Watsonville (CA)	121°46′07″	36°55′10″
MT1	Corvalis (MT)	114°05′46″	46°18′51″
MT3	Corvalis (MT)	114°01′54″	46°12′31″
OH1	Kingsville (OH) [†]	080°40′13″	41°55′02″
OH2	Wilmington (OH) [‡]	083°49′51″	39°26′37″
OR1	Corvallis (OR)	123°16′34″	44°34′16″
OR2	Milton-Freewater (OR)	118°23′25″	45°56′06″
NY1	Trumansburg (NY)	076°39′38″	42°32′25″
Europe (Switzerland)			
BE1	Ittigen (BE)	007°29′21″	46°58′55″
BE2	Flugbrunnen (BE)	007°30′27″	46°58′23″
FR1	Belmont (FR)	007°29′21″	46°49′54″
GE1	Commugny (GE)	006°09′35″	46°19′51″
GE2	Duillier-Gland (GE)	006°14′30″	46°24′37″
GE3	Duillier-Friedhof (GE)	006°14′06″	46°24′16″
VS3	Aproz (VS)	007°17′38″	46°12′22″
VS4	Vétroz (VS)	007°16′59″	46°12′22″

Australia

ME1	Tyabb (VIC)	145°09'45"	30°17'15"
ME2	Hastings (VIC)	145°09'10"	38°18'10"
ME3	Hastings (VIC)	145°11'25"	38°16'15"
ME4	Tynong (VIC)	145°39'25"	38°04'05"
ME6	Templestowe (VIC)	145°09'15"	37°44'30"
ME7	Arthurs Creek (VIC)	145°15'30"	37°33'22"
VI2	Warrandyte (VIC)	145°12'13"	37°44'59"
VI3	Warrandyte (VIC)	145°18'22"	37°45'32"

† collected from a vineyard

‡ collected from a raspberry crop; all other from apple orchards

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Appendix B. A summary of hierarchical mixed-model ANOVA (proc MIXED in SAS) in growth and reproductive traits of *Senecio vulgaris*.

TABLE B1. Summary of hierarchical mixed-model ANOVA (proc MIXED in SAS) in growth and reproductive traits of *Senecio vulgaris*. Values represent *F* ratios for fixed effects (rust treatment, region) and chi-square values for random effects (population nested within region, family nested within population). Block effects were also fitted, but are not shown here.

Source	Capitula number	Vegetative drymass	Secondary branches	Apical height	Reproductive allocation	Growth rate
treatment (t)	537.40 ***	753.85 ***	518.75 ***	827.11***	321.19 ***	54.85 ***
region	3.25 †	0.02	3.51 *	0.45	2.42	2.82 †
population	0.57	7.44 **	15.70 ***	16.27 ***	0.83	10.34 **
family	23.65 ***	72.28 ***	0	42.54 ***	0.68	29.70 ***
t × region	2.49	0.32	0.54	0.02	5.48 *	2.68 †
t × pop	17.78 ***	1.24	0	11.48 ***	7.88 **	3.33 †
t × fam	7.24 **	45.58 ***	2.82 †	3.61 †	0.40	0

†*P* < 0.10; **P* < 0.05; ***P* < 0.01; ****P* < 0.001

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Appendix C. A summary of hierarchical analysis of molecular variance (AMOVA) for 249 *Senecio vulgaris* individuals representing 24 populations from three regions: North America, Europe (Switzerland), and Australia.

TABLE C1. Summary of hierarchical analysis of molecular variance (AMOVA) for 249 *Senecio vulgaris* individuals representing 24 populations from three regions: North America, Europe (Switzerland), and Australia. The analysis is based on 143 polymorphic AFLP markers. Levels of significance are based on 10000 iteration steps.

Source of variation	Variance components			
	df	VC	%	<i>P</i>
Among region	2	0.66	6.43	< 0.001
Among population within region	21	1.83	17.80	< 0.001
Within population	225	7.73	75.67	< 0.001
Total	248	10.22	100	